Amendments to the Claims

Claims 1-53 (Cancelled)

Claim 54 (Currently Amended): A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein:

a) said loading module loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules; and

b) said loading module is of the form:

(engineered-KSq)-(AT)-(ACP), wherein:

i) ACP is an acyl carrier protein domain;

ii) AT is an acyltransferase domain which loads an optionally substituted malonyl; and

iii) engineered-KSq is a ketosynthase (KS) domain which ~~has been genetically engineered to~~ effects decarboxylation of a loaded optionally substituted malonyl ~~by mutating the active site cysteine residue to a glutamine residue~~, wherein said engineered-KSq domain is obtained by replacing the active site cysteine of a KS domain of an extension module with a glutamine; ~~and~~

~~c) at least the first of said extension modules is not naturally associated with said loading module;~~

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 55 (Previously Presented): A type I polyketide synthase according to claim 54, wherein said acyltransferase domain has an arginine residue in the active site.

Claim 56 (Previously Presented): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is a natural extension module acyltransferase domain.

Claim 57 (Previously Presented): A type I polyketide synthase according to claim 54, wherein the engineered-KSq and acyltransferase domain pair produced by mutation occur together in an extension module in their unaltered state.

Claim 58 (Previously Presented): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is specific for loading with malonyl.

Claim 59 (Previously Presented): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is specific for loading with methylmalonyl.

Claim 60 (Currently Amended): A type I polyketide synthase according to claim 55, wherein said acyltransferase domain is ~~selected from the group consisting of the acyltransferase domain of extension module 5 of the monensin polyketide synthase and~~ the acyltransferase domain of extension module 5 of the spiramycin polyketide synthase.

Claim 61 (Previously Presented): A type I polyketide synthase according to claim 56, wherein said acyltransferase domain is the acyltransferase of module 6 of the niddamycin polyketide synthase.

Claim 62 (Previously Presented): A type I polyketide synthase according to claim 56, wherein said acyltransferase domain is the acyltransferase of module 4 of the FK506 polyketide synthase.

Claim 63 (Currently Amended): A type I polyketide synthase according to claim 54, wherein said polyketide synthase is effective to synthesize a polyketide selected from

(a) 12- and 16-membered macrolides with acetate starter units;

(b) 12, 14, and 16-membered macrolides with propionate starter units;

(c) variants of rifamycin, avermectin, rapamycin, immunomycin and FK506 which differ from the natural compound in the incorporation of acetate starter units or propionate starter units;

(d) a polyketide wherein the starter unit is derived by the action of said engineered-KSq domain on the enzyme-bound product of ~~from a loading domain comprising the acyltransferase~~ said AT domain, wherein said AT domain is from ~~of~~ extension module 4 of the FK506 polyketide synthase; or

(e) a polyketide wherein the starter unit is derived by the action of said engineered-KSq domain on the enzyme-bound product of ~~from a loading domain comprising the acyltransferase~~ said AT domain, wherein said AT domain is from ~~of~~ extension module 6 of the niddamycin polyketide synthase.

Claim 64 (Currently Amended): A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein:

a) said loading module loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules; and

b) said loading module is of the form:

(KSq)-(AT)-(ACP), wherein:

i) ACP is an acyl carrier protein domain;

ii) AT is an acyltransferase domain which loads an optionally substituted malonyl and is selected from the group

consisting of the acyltransferase domain of module 6 of the niddamycin polyketide synthase, the acyltransferase domain of module 4 of the FK506 polyketide synthase, and ~~the acyltransferase domain of module 2 of the rapamycin polyketide synthase,~~ the acyltransferase domain of module 5 of the spiramycin polyketide synthase~~, and the acyltransferase domain of module 5 of the monensin polyketide synthase~~; and

iii) KSq is a domain which effects decarboxylation of a loaded optionally substituted malonyl and which differs from a ketosynthase domain of an extension module by having a glutamine residue in place of the cysteine residue in the active site; ~~and~~

~~c) at least the first of said extension modules is not naturally associated with said loading module;~~

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 65 (Previously Presented): The type I polyketide synthase according to claim 64, wherein (AT) is the acyltransferase domain of module 6 of the niddamycin polyketide synthase,

Claim 66 (Previously Presented): The type I polyketide synthase according to claim 64, wherein (AT) is the acyltransferase domain of module 4 of the FK506 polyketide synthase.

Claim 67 (Cancelled)

Claim 68 (Previously Presented): The type I polyketide synthase according to claim 64, wherein (AT) is the acyltransferase domain of module 5 of the spiramycin polyketide synthase.

Claim 69 (Cancelled)

Claim 70 (Currently Amended): A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein said loading module is of the form:

(KSq)-(AT)-(ACP), wherein:

i) ACP is the acyl carrier protein domain of the erythromycin loading module;

ii) AT is the acyltransferase domain of module 2 of the rapamycin polyketide synthase; and

iii) ~~The type I polyketide synthase according to claim 64, wherein the~~ KSq ~~domain~~ is the KSq domain of the oleandomycin loading module.

Claim 71 (Currently Amended): The type I polyketide synthase according to claim 64, wherein said extension modules are selected from the group consisting of extension modules from the erythromycin, rifamycin, avermectin, rapamycin, ~~immunomycin,~~ or FK506 polyketide synthases.

Claim 72 (Cancelled)

Claim 73 (Currently Amended): A type I polyketide synthase which produces a polyketide and which comprises a loading module and a plurality of extension modules, wherein:

a) said loading module ~~loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules;~~

~~b) said loading module is of the form:~~

~~(KSq) (AT) (ACP), wherein:~~

~~i) ACP is an acyl carrier protein domain;~~

~~ii) AT is an acyltransferase domain which loads an~~

~~optionally substituted malonyl; and~~

~~iii) KSq is a domain which effects decarboxylation of a loaded optionally substituted malonyl and which differs from a ketosynthase domain of an extension module by having a glutamine residue in place of the cysteine residue in the active site;~~

~~c) at least the first of said extension modules is not naturally associated with said loading module; and~~

~~d) said loading module~~ is the loading module of the monensin polyketide synthase; and

b) at least the first of said extension modules is not naturally associated with said loading module;

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 74 (Cancelled)

Claim 75 (Currently Amended): A type I polyketide synthase which produces a 12- or 14- membered macrolide and which comprises a loading module and a plurality of extension modules, wherein:

a) said loading module ~~loads an optionally substituted malonyl and then effects decarboxylation of the loaded moiety to provide a corresponding optionally substituted acetyl moiety for transfer to the first of said extension modules;~~

~~b) said loading module is of the form:~~

~~(KSq)-(AT)-(ACP), wherein:~~

~~i) ACP is an acyl carrier protein domain;~~

~~ii) AT is an acyltransferase domain which loads an optionally substituted malonyl; and~~

~~iii) KSq is a domain which effects decarboxylation of a loaded optionally substituted malonyl and which differs from a ketosynthase domain of an extension module by having a~~

~~glutamine residue in place of the cysteine residue in the active site;~~

~~c) at least the first of said extension modules is not naturally associated with said loading module; and~~

~~d) said loading module~~ is the loading module of the tylosin polyketide synthase; and

b) at least the first of said extension modules is not naturally associated with said loading module;

wherein the polyketide produced by the polyketide synthase is other than a 14-membered macrolide having a 13-methyl group due to incorporation of an unsubstituted acetate starter.

Claim 76 (Cancelled)